Lecture 4. Columbia University. April 15, 1964

Gene-Control Mechanisms in Higher Organisms. Part II.

I. Review of previous lecture:

1. Attempt to show the relation of cytoplasmic-gene interactions in control of cell differentiation as a consequence of control of specific genes.

2. Control of action of alleles: only one active at a time; of duplicate genes and multiples of such genes: only one active at a time; of regulators that determine the patterns of this; of two-element systems regulatin chromosome behavior with B-type chromosomes; of regulatory mechanisms controlling pattern of distribution of one product of gene action - elytra patterns in Lady Beetles.

3. The built-in Genetic Timers: in control of patterns of expression of particular genes; of phase variation -- active and inactive regulators and their cycles.

II. Levels of control mechanisms:

1. The model of Jacob and Monod: basic mechaism of control of gene action.

2. The higher levels: Consider controls responsible for several different types of organisms from one germplasm:

a). Larvae - adult in Drosophila; catipellar - moth; Parasites - several different organisms from one germplasm- How controlled? Major "switch" genes known.

b). Polymorphism in insects: Levels: From altered patterns of pigments, as in Lady Beetle, to mimicry. Mimicry can involve a series of changes in structure. Genetic analysis, indicates control by one or two "Switch" genes.

3. Genetic components in chromosomes that do nothing else but regulate gene action: Regulate when a gene system is active; Regulate the kind of product of gene action. Do this through system of two elements.

a). These systems of regulator: Not like "operator-regulator" systems in bacteria. They have "built-in" mechanisms that modulate the transcription of the gene: this resembles gene mutation changes. Type of change controlled. Several types may be produced during development: mechanism controls type at any one time.

b). These control mechanisms discovered in maize many years ago.

III. Discovery of pattern controlling mechanisms in maize: when a gene will be active and kind of product resulting from its action during development.

1. Initial experiment: chromosome type of b.f.b.cycle. Reason for exp.

2. How experiment conducted: [illegible]

3. Results of experiment: The selfed progeny - seedlings. The modified action of genes in somatic tissue: Slides 1 to 5.

4. Conclusions from observations: mechanism controlling how a gene acts and how changes in this occur: related to some event occurring during a mitotic cycle: questions: What is this mechanism? Why did the b.f.b cycle reveal these mechanisms? What is present in the maize genome that can become altered in such a manner that it will produce such types of control of gene action?

5. Answers to questions obtained: Cycle uncovered presence of controlling elements: Two elements to a control system. These behave like Operators and Regulators:

1) Consider situation of phages: Can be inserted at various loci in chromosome complement. When inserted, become a part of the bacterial chromosome.

2) Consider controlling elements, something like phages, can transpose from one location to another in the chromosome complement. This occurs at the time of chromosome replication.

3). Consider phage-like elements belonging to a system: one can become inserted at locus of gene: then, gene comes under control of this element. Other element, located elsewhere, acts like regulator to which element, adjacent to gene will respond by turning on-or -off action of the gene or modulating action of the gene.

4). The controlling elements: transposible elements; each in system of two : an operator component and a regulator component.

5). When op. element transposed to <u>any</u> gene, this gene now under control of the system to which the operator element belongs.

6). These two-element systems of gene control named after their regulators

Each regulator of a system readily identifiable.

7) Examples of meaning of this: Take single "wild-type" gene:

One plant, one cell: Op-1 inserted at locus of gene A - Gene under control of op-reg system 1

Another plant, one cell: Op-2 inserted at locus of gene A - This gene now under control of op-reg- system 2

When Op-1 at gene locus, control of gene action only by Reg 1; Regs. 2, 3, 4, do not alter op-1; Each op-reg- system highly specific.

III. The super-imposing Op-Reg systems, above, present in maize from all over the world. Not recognized as such in early genetic studies.

1. The first case leading to puzzlement, not clarity: The a_1 - Dt system.

2. The A_1 gene;located in chr. 3; in biosynthetic pathway leading to anthocyanin in kernel and plant.

3. Standard recessive, a_1; very old mutant. Stable with mutagenic agents: Tests of this.

4. Discovery of "gene" that would make it mutate: Dominant, initially located at end of short arm of chromosome 9:

5. Effect produced by this gene: caused a_1 to mutate in specific way: small A_1 streaks and few dots of A_1 in kernels.

Alleles: stable after mutation process: No like original A_1 - various different levels of gene activity among alleles, some completely recessive. Gene

Alleles: mostly completely stable with Dt. Released from control by the Dt. system.

6. Studies of Nuffer:

a). Hunt for Dt in strains of maize from S. America: Two stains with Dt:

b). Location of these Dt in chromosomes: One in chr. 6, Other in chromosome 7. Neither at end of short arm of chromosome 9.

c).Later, found transposition of the Dt element to new locations.

d). Found new case of Dt controll of A gene: pattern of mutation not the same. Shows in slides.

e). Must consider development of kernel:

Embryosac Pollen grain

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Embryo and endosperm: twins, genetically.

Mature kernel: Slides 6 to 8.

Cell lineages during development of kernel: Slides 9 to 11.

f) Nuffer: standard a_1 and new Dt controlled a - Nuffer, Slide 12
New a_1 - mutations in anther when Dt present. Slide 13
Standard a_1 and new a_1 alleles in plant: Slide 14
Mutants; change in state: Kernels, Slide 15.

IV. Earlier test of presence of inactive Dt in maize not showing Dt regulation

1. From b.f.b. experiment, decided inactive regulators present.
2. On this premise, should be able to recreate Dt by b.f.b.cycle
3. The experiment: Cross

Female constitution

Chr. 3 a_1/ a_1

Chromosome 9: normal

Male constitution

Chr. 3 a_1/ a_1

Chr. 9

Gametes produced by male:

Functional male gametes: with broken end.

4. Observations of kernels on ears from this type of cross: Found sectors in which Dt present: Slides 16 - 18
5. Reason why only dotts produced in kernel, and their spacing: State of operator at standard a locus: will give late mutations with Dt only.
6. Review of Dt system: standard a : completely stable; cant cause mutatio by X-ray, U.V., or mutagenic agents. when Dt not present.
b). Dt present in inactive phase in maize plants not showing it.

c) Type of response to Dt: depends on the state of the operator at a-standard locus.

d). This shown by tests of new Dt controlled $\underline{a}_1$ locus: Original response to Dt; mutant of operator - changed response like standard ~~Dtx~~ $\underline{a}_1$.

e). Dt, the regulator, can transpose from one location to another in chromosome complement without losing identity in process.

V. The Spm system: The Ac system

1. Similar basic mechanism: two elements, an operator and a regulator.
2. Insertion of op ~~af~~ this system at A_1 locus: Now, gene under control of system to which operator belongs:
3. Isolation of cases of insertion of operators at normal A_1 locus: independent occurrences:

Dt system	Ac system	Spm system
Standard a_1	a_1^{m-3}, a_1^{m-4}	a_1^{m-1}, a_1^{m-2}, a_1^{m-5}
New cases:	McClintock	McClintock
One by Rhoades		a_1^{m} Peterson.
Onde by Nuffer		

4. Types of genes that have come under control of these and other systems: All types; Those selected for study. Reason

5. Other systems isolated, not yet defined.

VI. The Spm system -- examine ~~A_1~~ again.

1. Spm, the Regulator: Reason for name: Given in first studies:

Reason: Gene, op of Spm system adjacent: Spm present, no gene action until change occurred, in response to Spm, that gave particular pattern of gene action. These "mutations" as operator removed at time of occurrence of mutation.

Spm absent: Gene active. Degree depends on state of operator.

2. States selected as described above: Illustration, same two ears shown on Monday: Slide 19
3. Other illustrations of states: Pigments in kernels: Slides 20, 21

The independent control by each operator of time and type of gene action during development: Like Elytra of Lady Beetle.

State ~~5719x~~ 5719A-1 Slide 22; State5720 Slide 23
Cross: Slide 24. (sh_2)
Both states in same kernel with Spm: Slide 25

VII. Methods of testing for Spm and for response of gene locus to Spm

1. To test for Spm: with any change at A_1 locus: Tester stock:
State:5719A-1 Slide 26: Pr. Appearance with pr. pale

Spm

McSpm

Slide 27 : to show deep red with normal pr and A_1

2. Get stock with no Spm (completely inactive) this $a_1{}^m$ sh_2 pr, etc.

3. Plant: near colorless Sh_2 kernels on ear of an $a_1{}^m Sh_2/a_1$ sh_2 Spm plant
   a) Question: is Spm present in kernel and embryo?
   if not, would a gene respond to Spm? A changed state? a stable mutant?

   b) The tests: Grow plant from kernel. Cross ears:

   (1) by a_1 sh_2 no Spm For control of other tests.

   (2) by a_1 sh_2 Spm To test response to Spm

   (3) by $a_1{}^m$ sh_2 pr, no Spm: To test for presence of Spm.

   Slide 28: Three ears of plant 8424C-1 (Pr/Pr)

4. Take variegated kernels on ear in middle: have Spm.
   a). Grow plants from these kernels: Test for Spm distribution in progeny: The test:
   (1) Ear x a_1 sh_2 - for control
   (2) Ear x a_1m sh_2 pr
   Expect segregation for Pr and pr in test (2):
   Slide: 29 Two ears, plant 8939C-4.

5. Constitution of the plant being tested: wx/wx standard.(no response to Spm). Constitution of tester with $a_1{}^m$ = wx-m8: responds to Spm
   Gives series of alleles of Wx gene action.
   The Wx gene action: I-KI solutions (Demonstration)

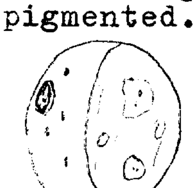
6. Pigment: in aleurone layer; starch, in cells under this layer.
   Expect two types of kernels with respect to wx gene action:
   No Spm: near wx in staining.
   Spm: sectors showing alleles of Wx gene;

   The Test: Slides 30, 31.

7. Illustration: Control of action of two very different genes by same system.

VIII Activation cycles of Spm:

1. How seen easily: Selected state: Operator fixed in location: no losses to locus -- no mutations -- no stabilization.
2. Effect produced: Normal wild-type action when no Spm present or when Spm inactive. When Spm present, gene inactive.
3. Shows changes in phase of activity of Spm: Active, colorless, inactive pigmented. Patterns expected from inactivation cycles:



Colored area -> colorless within -> colored within
[illegible] inactive Spm active Spm. cell lineages
Slides 32-34.

IX. Reversal of response of operator to Spm: One case isolated:

Spm active: gene active; Spm inactive: gene inactive.

Activation cycles: Slides 35; Test with wx^{m-8}: Slide 36.

Reason for dots of deep color: represent transpositions of Op away from locus of gene: release from control by Spm system.

Start with fully active Spm: Pattern observed: Slide 38: Time of transposition of Op from locus of A gene.

Lecture - 4.

Genetic mechanisms controlling gene action in higher org. 1

Slides -

1. to 5 - leaves - b.f.b. expt exp.

6. cross section of leaf - diagram.

7. Photo - [illegible], [illegible], [illegible]

8 " same enlarged

9 - Kernels - all lineages - C.S.H. Symp. 1951

10 - [illegible] - medium lines + late dark. +

11 " - very late [illegible] -

12 " - [illegible]. Fig 3

13 [illegible] " Fig. 4

14. [illegible] " Fig 1.

15 " " Fig. 6 - Enlargement - a, w + Ds. [illegible] - [illegible]

16 Kernels - normal Ds - Recessive Ds.

17 2 " - recessive Ds

18 Kernels - colored - normal Ds + " "

19 - Two [illegible] - Spm system

20 - colored ear - [illegible] pattern Pr

21 " " " " pr

22 [illegible] 5720 a, w^t

23 " 5719A-1 "

24 " " + 5720. Pr 2

25 " combined.

26 - [illegible] 5719A-1 Pr

27 " 5896-(4) pr

28 3 ears - 8424C (1)

29 2 " - 8439C-4.

30 1 " of " " & 8384B (2) [illegible]

~~31 - Same - enlarged~~ OK

32 - Slate - class II - 3 pure cycles - 6 leaves

33 " " " " 1 leaves - b. r white

34 same - colored

35. $a, \underline{\underline{m-2}}$ [illegible] → [illegible] leaves

36 " " " " [illegible]

37 " - Spm^{u}, Spm^{s}, Spm^{u} - colored leaves

38 - Stabilizer [illegible].